EFFECTS OF MAREK'S DISEASE HERPESVIRUS AND TURKEY HERPESVIRUS UPON IMMUNITY TO *EIMERIA ACERVULINA* IN YOUNG CHICKENS

An abstract of a Thesis by
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The problem. Concurrent infections with Marek's disease (MD) and coccidiosis in chicken flocks have perplexed investigators for years. Infection with MD herpesvirus has been shown to inhibit immunity of chicks to three species of *Eimeria*. Herpesvirus from turkeys (HVT) is used commercially as a vaccine against MD. This investigation studied the response to primary and secondary exposures to *E. acervulina* oocysts in young chicks infected with either MDHV or HVT.

Procedure. Groups of chickens were inoculated with either MDHV or HVT. These chicks received a primary immunizing series of inoculations with *E. acervulina* oocysts. Individual chick weights were recorded during this period. The chicks were subsequently challenged with a massive dose of *E. acervulina* oocysts. Following challenge chicks were monitored for weight gain or loss and for the number of oocysts passed in the feces. Five to seven days following challenge all chicks were killed and autopsied to evaluate for *E. acervulina* and MD lesions. Data from these chicks were compared to data from control chicks which received oocysts but no virus.

Findings. Weight gains, oocyst recovery data, and autopsy observations for experimental and control chicks are equivalent.

Conclusions. Neither MDHV nor HVT had any effect upon the immune response of young chicks to *E. acervulina*. Vaccination of chicks with HVT did not jeopardize development of resistance to *E. acervulina*.

Recommendations. Further studies with other strains of MDHV or with other species of *Eimeria* would be helpful in better understanding the relationship of MD and coccidiosis.

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by

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INTRODUCTION AND LITERATURE REVIEW

Coccidiosis is a disease resulting from infection with coccidia, protozoan parasites of the subphylum Sporozoa. The coccidian parasites of chickens belong primarily to the genus Eimeria, but may include other genera such as Isospora, Wenyonella and Cryptosporidium. These parasites normally invade cells of the intestine in which they undergo development. Coccidiosis in chickens may result in severe acute infections followed by high mortality or in chronic sublethal infections accompanied by a slower rate of weight gain, lower feed efficiency and reduced egg production (Brackett and Bliznick, 1950).

Eimeria acervulina Tyzzer (1929), is one of the more common coccidian parasites of poultry. It is considered to be only slightly pathogenic, but chickens inoculated with massive doses of E. acervulina oocysts may show considerable morbidity and mortality (Moynihan, 1950; Morehouse and McGuire, 1956). Common symptoms associated with E. acervulina infections in the host include weight loss, lowered egg production and a general droopy appearance. At necropsy, lesions resulting from infection with E. acervulina appear as whitish patches beneath the surface of the mucosa in the upper half of the small intestine (Lund and Farr, 1965). These whitish patches contain developing oocysts.

Marek's disease (MD) has been known as neural leukosis,

range paralysis and acute leukosis. MD may be confused with, and should be differentiated from, lymphoid leukosis which is also known as big liver disease, visceral leukosis, or chronic leukosis. These two diseases comprise the avian leukosis complex.

The first published account of MD was by Marek (1907) in Hungary. Lesions of MD involve proliferation of lymphoid cells into the nervous system and other organs and tissues. Macroscopic lesions involving the nervous system are most easily seen in the peripheral nerves. Affected nerves appear edematous with yellow or gray discoloration. Any nerve can be affected but the enlargement is best demonstrated macroscopically in the brachial plexus and nerve trunk, sciatic plexus and nerve trunk, coeliac plexus and vagus nerve. Nonneural tumors occur most commonly in the gonads but can be found in any organ or tissue. These lesions appear as non-lobed enlargements of the affected tissues with blotchy discoloration. Other common sites for MD tumors are in the lungs, kidneys, liver, heart, mesenteries, muscles, spleen, and skin tissues (Biggs, 1967).

Microscopically, lesions of MD in both nerve and lymphoid tumors are similar in appearance. Proliferation of lymphoid cells including primarily small lymphocytes with fewer numbers of medium sized lymphocytes, blast cells and plasma cells is characteristic of these lesions (Biggs, 1967).

Until recently, the etiology of MD was unknown. It had been shown that MD could be transferred experimentally from infected to uninfected chickens by direct physical contact (Biggs and Payne, 1963), through contaminated litter, by oral washings and feces (Witter and Burmester, 1967) and by air borne routes (Sevoian et al., 1963). It was suspected that MD was caused by a virus, but this had not been proven.

Because of the intracellular nature of the agent causing MD, early attempts to isolate it were unsuccessful. It was not until the late 1960's that a herpes type virus was found in MD tumors induced experimentally (Churchill and Biggs, 1967; Ahmed and Schidlovsky, 1968; Schidlovsky et al., 1969; Nazerian et al., 1968). Passage of these herpesvirus infected tissues or cell cultures readily produced MD, but early attempts at cell free transmission were unsuccessful. Cook and Sears (1970) extracted infective viruses from cell cultures by treatment of infected chick embryo fibroblasts with demineralized water. At about the same time Nazarian and Witter (1970) recovered MD virus from the feather follicles of chickens infected with MD. This virus proved to be infectious when prepared in cell free extracts.

Isolation of the herpes virus causing Marek's disease (MDHV) opened the way for the development of vaccines for the control on MD. Churchill et al. (1969) successfully immunized young chickens against challenge with MDHV by vaccinating them with a tissue culture attenuated strain

of MDHV.

The rush to develop attenuated MDHV vaccines was short lived. Witter et al. (1970) isolated a cytopathic herpes virus in chick kidney cell cultures and in duck embryo fibroblast cell cultures from blood and kidneys from turkeys. This herpes virus isolated from turkeys (HVT) was shown to be serologically related to MDHV but was pathogenic to neither turkeys nor chickens. Subsequent work demonstrated that chickens vaccinated with HVT were protected against virulent MDHV (Okazaki et al., 1970a). Later work showed that vaccination with HVT before exposure to MDHV was important to development of immunity in the chicken (Okazaki et al., 1970b). Vaccination of chicks with HVT at one day of age has become a standard practice in many commercial hatcheries.

Concurrent outbreaks of MD and coccidiosis in chicken flocks are common. For almost half a century poultry researchers have worked to determine if a relationship exists between MD and coccidiosis, and, if so, to determine the nature of this relationship. One early report considered MD to be a chronic form of coccidiosis (Beach and Davis, 1925). While later efforts demonstrated that these two diseases were separate, their interaction was not understood until recently. Hess (1963) showed statistically that the frequency of concurrent infections with MD and coccidiosis is greater than that which would occur by chance alone.

In the late 1960's, investigators at the Houghton Poultry Research Station, Huntington, England conducted an extensive testing program to determine the nature of the relationship between MD and coccidiosis (Long et al., 1968; Biggs et al., 1968; Biggs et al., 1969). Attempts to transmit MD via coccidial oocysts were unsuccessful. These investigators were able, however, to demonstrate that MD virus infection impairs the immunological capacity of chickens. Further work demonstrated that MD increases the susceptability of chickens to coccidiosis and decreases the ability of chickens to develop immunity to coccidiosis. The coccidia used in these studies are E. mivati and E. maxima.

These observations have been confirmed by other investigators. Burg et al. (1971) showed that MD in chickens results in impairment of both the thymus and bursal dependent immune systems. Rice and Reid (1973) demonstrated suppression of immunity to E. tenella and E. maxima in chickens infected with MD. This suppression was greater when MD virus was administered at one day of age than when it was given at 28 days of age.

The present study was an investigation of the effects of MDHV and of current vaccination programs using HVT vaccine upon resistance of young chickens to the coccidium Eimeria acervulina.

MATERIALS AND METHODS

148 one-day old white leghorn female chicks (Hy-line production hybrid line 934-E) were obtained from the Blue Ribbon Hatchery, Indianola, Iowa. The chicks were from dams with no history of MD or of vaccination with HVT. These chicks were not tested serologically for MD antibody. The chicks were divided into four groups of 30 chicks each and one group of 28 chicks. Each chick was identified with a letter (A, B, C, D, or E) corresponding to the group to which it was assigned and with a number (1 through 28 or 30) representing the individual within its group. Group B contained only 28 chicks.

Each group of chicks was caged separately in a 2' by 3' by 10" high brooder unit containing a heater at one end. The heaters were set to maintain relatively constant temperatures of about 95° F. at chick level, directly under the heater unit. The heaters were shut off after 10 days. At about 14 days of age, the chicks were becoming crowded in these brooder units so each group was transferred to a separate 3' by 4' by 12" high cage. Late in the study each group was split to facilitate data collection as will be discussed later. Chicks numbered 1 through 15 in each group were divided into 3 sets of 5 chicks each. At that time a second 3' by 4' by 12" high cage for each group was partitioned with wire screening into three 3' by 16" by 12" high

sections, one for each set of 5 chicks. The remaining chicks in each group were kept in the original cages.

All cages and brooder units had wire floors, removable dropping trays and detachable water and feed trays which hooked onto the outside of the unit.

From the beginning of the experiment the brooder units and cages were housed in isolation rooms designed to minimize the spread of airborne agents. Groups A, B, and C were housed in separate isolation rooms and groups D and E were housed in a fourth isolation room. Groups D and E were still maintained in separate cages and treated separately, however. Each isolation room was individually equipped with its own water supply, sewer drain, temperature control and ventilating system. Since this experiment was conducted in the early fall an auxiliary heating system which was available was not used, and the temperature remained ambient.

Each isolation room had both an air intake and exhaust system. Incoming outside air was passed through a Farr HP300 bacteriological filter system before being channeled into the rooms. A slight negative pressure was maintained in the isolation rooms relative to their connecting corridor to minimize the passage of air and airborne agents from the rooms.

The isolation rooms were constructed with concrete floors, cinder block walls and pressboard ceilings. The walls and ceilings had been coated with an epoxy type paint

in order to fill-in existing cracks and pits. Prior to beginning this experiment the equipment, walls, ceilings and floors of each room were cleaned with an aqueous solution of Environ-D detergent and disinfectant (Vestal Laboratories) using a high pressure spray gun and stiff brushes. There had been no animals in any of the isolation rooms for 10 weeks prior to the beginning of this experiment.

To be sure that all chicks within each group had equal opportunity to eat and drink, the feed and water trays were not allowed to go empty except near the completion of the study as noted later. Throughout the experiment all chicks were fed a custom mixed feed consisting of weight of 1 part wheat middling, 7 parts ground corn and 4 parts chick grower-developer concentrate (Supersweet #0203). This mix was prepared at a local feed mill and bagged so that each isolation room contained a separate feed supply. This feed mix was non-medicated and was not sterilized.

In caring for the chicks each room was entered only once each day except in the final days of the study when fecal samples were being collected. While caring for a group of chicks the feed trays were refilled, the water trays were emptied, rinsed and brushed if necessary and refilled, dropping trays were scraped and rinsed, and chicks were weighed if scheduled. Feces from the dropping trays was washed down the floor drains.

Until the chicks were 13 days old group B was always

cared for last because it was the only group which had received any experimental treatment. Groups A, B and C were all receiving experimental inoculations after 13 days of age. After that the groups of chicks were cared for in the order E, D, C, B and A. This order presented the least possibility of carrying coccidial oocysts or virus from an inoculated group to an uninoculated group. Treatment of groups is discussed later in this section.

In order to reduce the possibility of contamination by carrying oocysts and/or viruses between groups, the scales were wiped with paper toweling after weighing each group and were sprayed with Lysol disinfectant before each day's use. Also, hands were rinsed with water before and after handling and caring for each group. To reduce the possibility of contamination by insects, Shell "no pest strips" were placed throughout the building and the corridor connecting the isolation rooms was sprayed periodically with Black Flag Fly Spray.

Chicks used during this experiment were not exposed to other chickens except at the hatchery or to other facilities where they could have been exposed to extraneous coccidial infections. The author was not in contact with any other chickens during the study. When it was necessary for someone else to care for the experimental chicks they did so early in the day before coming in contact with other chickens.

The oocysts of E. acervulina used in this study were

obtained originally from Salsbury Laboratories, Charles City, Iowa. According to Dr. Ted Rude of Salsbury Laboratories, these oocysts represented a clone from a single oocyst which his staff had isolated from a naturally infected chicken.

In order to obtain a larger quantity of oocysts for additional inoculum, the oocysts received from Salsbury Laboratories were passed once in young cockerels. These cockerels were hatchmates of the experimental chicks and were caged separately, but in the same room as group C. Ten of these cockerels were inoculated with 500,000 oocysts each at 14 days of age. Oocysts were inoculated by intracrop cannulation using a four inch, twelve gauge stainless steel cannula attached to a 1 cc tuberculin syringe. 24-hour accumulations of feces were collected from these chicks on the 4th, 5th and 6th days following inoculation of oocysts. No oocysts were recovered from the feces collected on the 4th day. The identity of the recovered oocysts was not confirmed serologically but they were compatible with E. acervulina with respect to prepatent period, size, shape and sporulation time (Lund and Farr, 1965; Morehouse and McGuire, 1958).

Oocysts which were shed were separated from the feces by the following sodium chloride flotation method modified from Baron and Morehouse (1963). One volume of fecal material was combined with 2 volumes of saturated sodium chloride solution and mixed in a Waring blender. This

suspension was then distributed in several 250 ml centrifuge bottles which were placed in a swinghead centrifuge (International model K2) and spun at 2000 rpm for 5 minutes. The supernatant was poured into a beaker and saved and the sediment was resuspended in saturated sodium chloride solution and centrifuged again. The supernatant was again poured off into the beaker containing that from the first centrifugation. The sediment was then discarded. One volume of the supernatant was then diluted with five volumes of water and centrifuged in order to remove the oocysts from suspension by sedimentation. The supernatant was discarded and one volume of sediment containing the oocysts was suspended in approximately 50 volumes of 2.5% potassium dichromate. This suspension was poured into Petri dishes to a depth of 2 mm, covered, and the oocysts allowed to sporulate at room temperature for 24 hours. The sporulated oocysts were separated from the potassium dichromate by centrifugal sedimentation and resuspended in physiological saline. The number of oocysts per ml of physiological saline was determined by placing a sample from a freshly mixed suspension of oocysts under the cover glass of a hemocytometer (American Optical Company, Spencer Bright Line) and with the aid of a compound microscope equipped with a 10x objective and a 10x occular, counting the number of sporulated oocysts distributed over the grid. The number of sporulated oocysts per ml of physiological saline was determined by the following formula:

$$\frac{\#O.C.}{\#L.S.C.} \times 9 \times \text{dilution factor} \times 10^4 = \#\ \text{oocysts/ml}$$

O.C. represents the number of oocysts counted, L.S.C. is the number of large squares of the hemocytometer grid counted, 9 is the number of large squares on the grid, and 10^4 converts the volume from 0.1 cubic mm to 1 ml. If it was not necessary to dilute the sample, the dilution factor would be 1. Oocysts were then diluted with additional physiological saline to make suspensions of 75,000, 100,000, and 5,000,000 oocysts per 0.5 ml to be used later for inoculum.

Salsbury Laboratories also supplied the JM strain (Sevoian et al., 1962) of MDHV used in the present study. The virus is identified by Salsbury code number JM3530-5 and had been passed 6 times in chick embryo fibroblast cells following its original isolation from infected chickens. The virus titer was indicated to be 25,000 focus forming units per ml. The virus was stored in sealed glass ampuoles in liquid nitrogen until used. The quantity of virus received was sufficient for the entire study and further passage was not necessary.

Of the five groups of chicks, two (A and B) were experimental. For group A, each chick received 0.2 ml of MDHV virus injected at 13 days of age. The chicks were inoculated subcutaneously with the aid of a tuberculin syringe coupled to a 1 inch, 22 gauge hypodermic needle beneath the loose skin on the inside of the thigh near the abdomen. For group B,

each chick was vaccinated according to the instructions of the vaccine manufacturer subcutaneously beneath the loose skin on the back of the neck with 0.2 ml of HVT vaccine (Sterwin Laboratories, Inc.) one day after hatching with the aid of a Cornwall automatic pipetting syringe coupled to a ½ inch, 22 gauge hypodermic needle. The other three groups (C, D, and E) were designated as control groups.

Groups A and B and control group C also received a series of inoculations with oocysts of E. acervulina. Each chick in these three groups received 25,000 oocysts daily from the 15th through the 19th day after hatching. On the 20th through the 24th day after hatching the dosage was increased to 50,000 oocysts daily per chick. The oocysts given to this point were directly from the original stock received from Salsbury Laboratories. All subsequent oocyst inocula were prepared from the original stock as described earlier. On the 27th through the 31st day each chick received 75,000 oocysts daily. The highest dosage, 100,000 oocysts per chick, was given daily from the 32nd through the 36th day after hatching. This regimen was followed on the advise of Dr. Rude and his associates of Salsbury Laboratories, and is considered by them to adequately induce immunity to E. acervulina in normal chickens.

At 43 days of age, chicks in groups A, B, and C and the second control group (D) were given 5,000,000 oocysts of E. acervulina each. This dose served as a challenge for

groups A, B, and C.

Group C received identical inocula of E. acervulina oocysts as groups A and B but received neither virus. Group C was therefore an experimental control. It is assumed that, if chicks in groups A and B had not received MDHV or HVT inoculations, they would respond to E. acervulina exactly like the chicks in group C. Group D was included to measure the potency of the challenge dose of E. acervulina oocysts administered to chicks in groups A, B, and C. Chicks in group E received no treatment at any time, and served as normal controls. Table 1 summarizes the groups and the treatment they received.

Table 1. Summary of groups and treatment. "Yes" indicates treatment was given. "No" indicates treatment was not given.

	TREATMENT			
GROUP	JM strain MDHV	HVT vaccine	E. acervulina Immunized	E. acervulina Challenged
A	yes	no	yes	yes
B	no	yes	yes	yes
C	no	no	yes	yes
D	no	no	no	yes
E	no	no	no	no

Weight loss or a reduced rate of weight gain is a classical symptom of infection with E. acervulina (Lund and Farr, 1965; Morehouse and McGuire, 1958). All chicks were weighed regularly during the course of the experiment. Chicks were weighed individually on a Chatillon spring type autopsy scale model 1309ADD. All weights were recorded to the nearest 5 grams. Chicks in all five groups were weighed individually at 15, 18, 21, 24, 27, 30, and 33 days of age. Chicks in groups A, B, and C were receiving daily inoculations with E. acervulina oocysts during that period of time.

Following inoculation of groups A, B, C, and D with the challenge dose of E. acervulina oocysts, all five groups were divided as follows. Chicks numbered 16 and higher in each group were maintained in their original cages and were weighed daily until killed for autopsy. Chicks numbered 1 through 15 in each group were divided into 3 sets of 5 chicks each (1 through 5, 6 through 10, and 11 through 15) for purposes of collecting feces and determining the number of oocysts excreted per bird following challenge with E. acervulina oocysts. The sets of 5 chicks were recaged as described earlier. Beginning on the 4th day after challenge with E. acervulina oocysts, 24-hour accumulations of feces were collected for each set of chicks from the dropping trays. The oocysts were separated from the feces and counted as described previously. The number of oocysts passed per set of 5 chicks for each 24-hour period was divided by 5 to give

the number of oocysts passed per chick. Fecal collections were made daily until the birds were killed for autopsy.

In order to prevent excess feed from being scattered into the dropping trays from the troughs and complicating oocyst isolation, the sets of 5 chicks evaluated for oocyst discharge were fed a limited quantity of feed during periods of oocyst collection. A one pint container of feed was given twice daily for each set of 5 chicks. Water was not limited. Because of the limited feeding these chicks were not weighe during the final phase of the experiment.

All chicks were autopsied on the 49th or 50th day after hatching and were examined for lesions of MD and E. acervulina. At autopsy the viscera, vagus nerves, and sciatic nerves were examined macroscopically for lesions of MD and the small intestine of each chick was opened longitudinally and examined for lesions. E. acervulina lesions were scored on a scale of 0 to 4 as described by Reid and Johnson (1970) with modifications. The criteria for scoring these lesions are indicated in Table 2.

For purposes of evaluating the effects of MDHV and HVT upon the chicks in this study, the weight data, oocyst recovery data and autopsy data from groups A and B were compared to group C. Since actual chick weights were not as significant as the weight gain or loss (Δwt) between weighings, the weight data was analyzed in terms of Δwt. Student-t tests were performed to determine the significance of

variations of data of groups A and B from group C relative to variations within the groups. Correlation at a probability of 0.95 was assumed to indicate no difference between groups.

Table 2. Criteria for scoring of lesions of E. acervulina.

SCORE	DESCRIPTION OF LESION
0	No lesions, and absence of oocysts in mucosal scrapings from intestine.
1	Scattered white focal lesions limited to the mucosa of the duodenum with a maximum of five lesions per square centimeter; oocysts present in mucosal scrapings from intestine.
2	Lesions more numerous but not coalescent, and extending as far as 20 centimeters below the duodenum.
3	Coalescent lesions giving the intestinal mucosa a white coated appearance with the intestinal wall thickened and contents watery; lesions may extend as far as the yolk sac diverticulum.
4	Coalescent lesions with areas of hemorrhage; intestinal wall greatly thickened with contents consisting of a white creamy exudate. Birds dying of coccidiosis were scored as 4.

Equipment and facilities for this study were provided through the courtesy of Hy-line Poultry Customer Service Laboratory, Dallas Center, Iowa.

DATA AND DISCUSSION

The purpose of this investigation was twofold: (1) to determine if MDHV infection in chickens produces an increased susceptability to E. acervulina similar to that described for E. mivati, E. maxima, and E. tenella (Biggs et al., 1969; Rice and Reid, 1973), and (2) a parallel study to determine if chickens vaccinated against MD with live HVT are more susceptable to E. acervulina than are unvaccinated chickens.

No differences were detected between the responses of chicks given MDHV (group A) and chicks vaccinated with HVT (group B) from the experimental control chicks (group C) to the series of inoculations with E. acervulina oocysts given from day 15 through day 33. Weights recorded during that period are listed in the Appendix in tables 3 through 7 representing groups A through E respectively. Figure 1 presents mean weight gain or loss (Δwt) values calculated from those weights. When the Δwt values of groups A and B are compared to group C using the student-t test, both groups correlate well with group C.

Groups A, B, and C had reduced Δ wt's as compared to uninoculated groups D and E on days 21 and 24 followed by a dramatic increase in Δwt by day 27. Following the first few oocyst inoculations, chicks in groups A, B, and C became very droopy with ruffled feathers and moved with slow jerky motions. This condition lasted from day 19 through day 23.

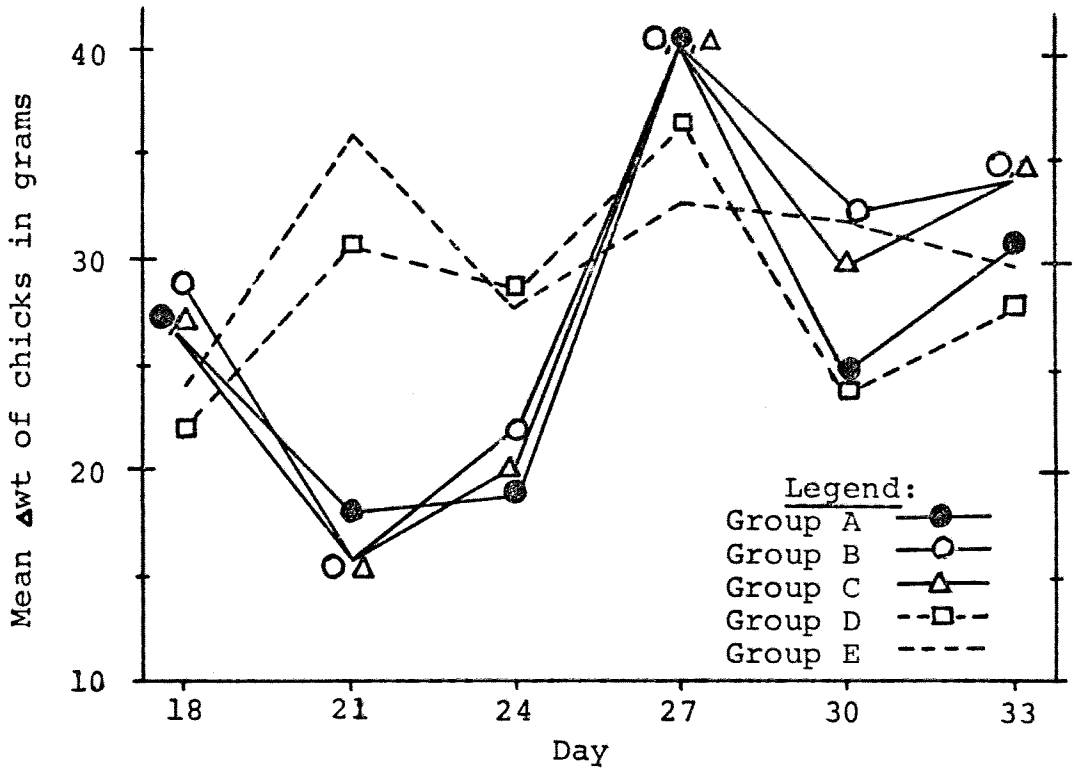

Figure 1. Mean Δwt values of chicks calculated from tables 1 through 5 in the Appendix. Chicks in groups A, B, and C received a series of inoculations with oocysts of E. acervulina beginning on day 15. Groups D and E received no oocysts at this time.

By day 25 all chicks in groups A, B, and C appeared and acted normal again. From day 25 until the completion of this portion of the study on day 33, these inoculated chicks could not be distinguished from the uninoculated chicks in groups D and E on the basis of appearance, actions or Δwt.

It can be shown from the data that, although the chicks in groups A, B, and C received oocyst inoculations from day 15 through day 33, the disease had run its course by day 24. Following a prepatent period of 4 days the chicks in groups A, B, and C developed symptoms of severe infection with E. acervulina. By day 24 these chicks were able to develop a high enough level of immunity to overcome their coccidiosis and to resist infection from further inoculations with E. acervulina.

There appears to be little difference in the effects of the challenge dose of E. acervulina oocysts upon chicks in groups A, B, and C. The effects of this challenge dose of oocysts upon chicks in groups A, B, and C were minimal because of the immunity they developed in the course of their previous exposure to E. acervulina. In contrast, the effects of this inoculation of oocysts upon the chicks in group D which had not previously received oocysts were predictably severe.

Chick weight data taken following administration of the challenge dose of E. acervulina oocysts to groups A, B, C, and D is recorded in tables 8 through 12 in the Appendix

for chicks in groups A through E respectively. Mean ▲wt values for that period are presented in figure 2. From figure 2 it is seen that mean ▲wt values for groups A, B, and C are within the same range as the values for group E which received no oocyst inoculations. It is evident, therefore, that these oocysts had no measurable effect upon the ▲wt of the chicks in groups A, B, and C and that they were immune to E. acervulina as a result of their primary exposure. A student-t analysis indicates good correlation of ▲wt data from groups A and B each with group C.

Figure 2 also illustrates the effects of the E. acervulina challenge dose upon the nonimmune chicks in group D. The dip in mean ▲wt for group D parallels a period of overt symptoms of E. acervulina infection as described earlier followed by apparent recovery by day 49. One chick in group D died on day 48.

The results of oocyst isolations from feces collected from sets of 5 chicks from groups A, B, C, and D on days 48 and 49 are presented in the Appendix in table 13. Fecal samples collected on day 47 yielded no oocysts. Also, no oocysts were isolated from any fecal samples collected from the sets of chicks in group E.

Figure 3 presents graphically the mean number of oocysts isolated per chick calculated for groups A, B, C, and D. As was expected, the oocyst production from the nonimmune chicks in group D was much greater than from the

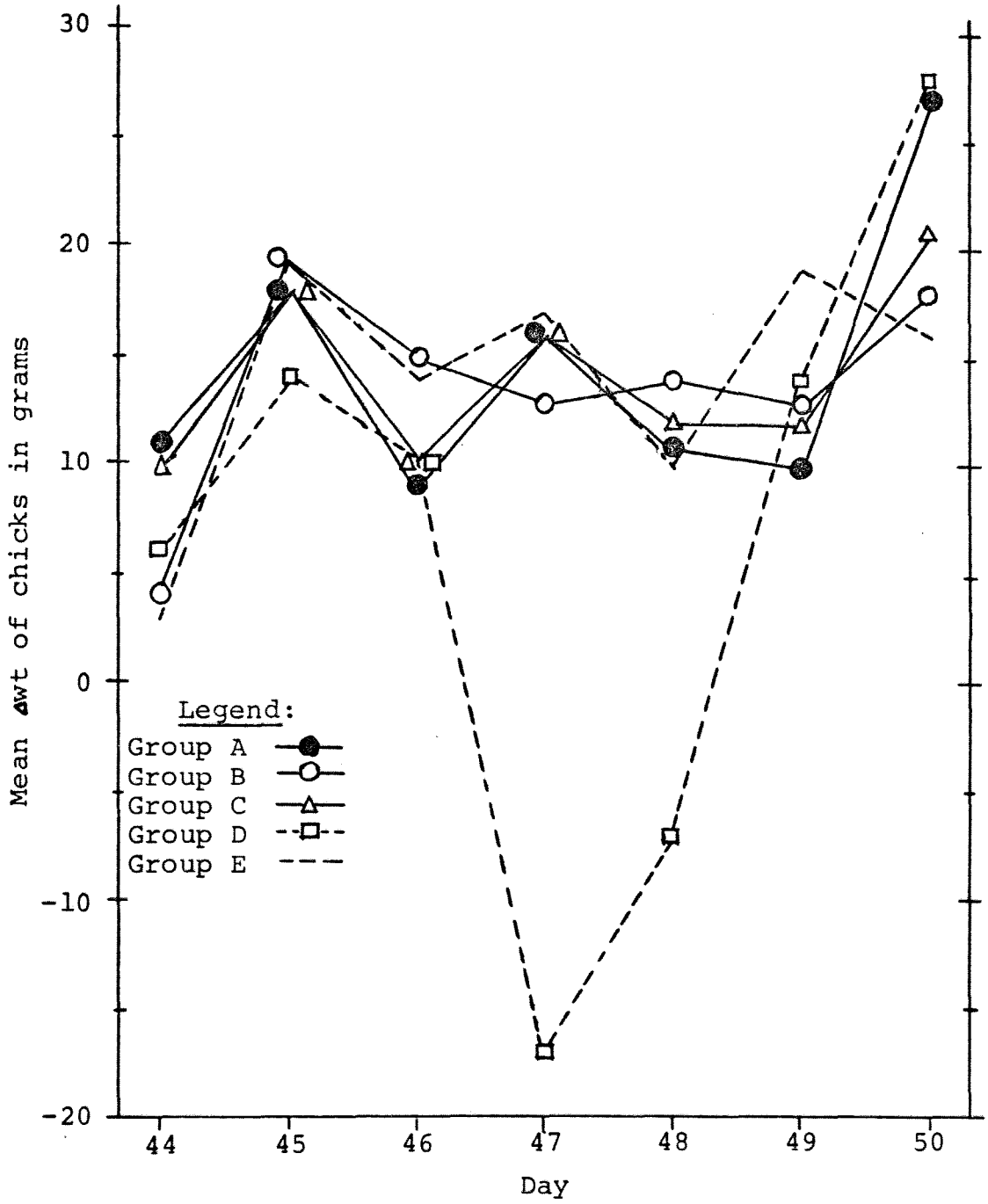

Figure 2. Mean Δwt values of chicks calculated from tables 6 through 10 in the Appendix. On day 43 chicks in groups A, B, C, and D received an inoculation of 5,000,000 E. acervulina oocysts each. Group E received no oocysts.

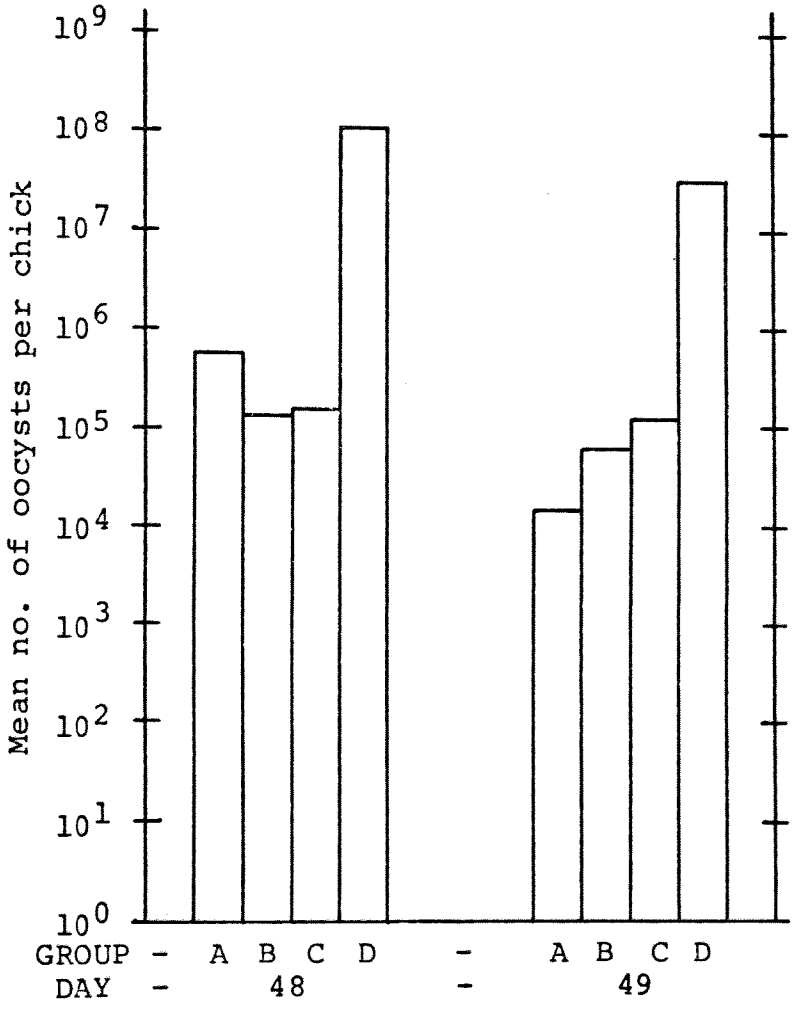

Figure 3. Mean number of oocysts isolated per chick for groups A, B, C, and D. All four groups received an inoculum of 5,000,000 E. acervulina oocysts per chick on day 43. Groups A, B, and C were previously exposed. Group D was nonimmune.

previously exposed chicks in groups A, B, and C.

If MDHV or HVT had reduced the ability of chicks in groups A or B respectively to withstand E. acervulina challenge, the result would have been higher oocyst production for those chicks than for the chicks in group C. From figure 3 it is apparent that group A did have a higher oocyst production per chick on day 48 than did group C. A student-t test of the data, however, indicates that this difference is not great enough to be significant.

Information regarding lesions of E. acervulina and MD observed at autopsy is presented in table 14 in the Appendix. There were no abnormalities noted in any of the chicks except those attributed to either MD or E. acervulina.

As was expected, E. acervulina involvement was greatest in the nonimmune chicks in group D. Group E which was not exposed to E. acervulina at any time showed no evidence of coccidiosis.

All chicks in groups A, B, and C were sufficiently resistant to E. acervulina as a result of the previous series of oocyst inoculations to prevent lesion formation following challenge. No E. acervulina lesions were seen in any chicks from groups A, B, or C. One chick in group B was, however, scored as 1 based on oocyst recovery in a scraping. There is no indication that either MDHV or HVT rendered chicks more susceptable to E. acervulina lesion formation following challenge than were the control chicks in group C.

No MD lesions were seen in any chicks other than those in group A. Although all chicks in group A had received MDHV inoculations, only 5 chicks contained demonstrable lesions. Those lesions were all early neural lesions. No chicks in this study exhibited lameness or paralysis typical of MD. Although the Hy-line 934-E hybrid chickens are bred to be highly resistant to MD, it had been expected that MD involvement in the chicks in group A would have been greater than it was.

Based on weight gains, oocyst recovery, and autopsy observations, neither HVT nor MDHV had any effect upon the immune response of chicks in this study to E. acervulina. Further studies could, however, shed more light upon the effects of MDHV and HVT upon resistance to E. acervulina.

If this study is repeated a less extensive primary exposure to E. acervulina may yield a more equivocal immunity to a secondary exposure. It is possible that the protracted series of oocyst inoculations used here was so overwhelming that minor variations in immune competence between groups of chicks were overcome. A lighter primary antigenic stimulus could bring out small differences between the groups in their ability to resist challenge. Perhaps 3 or 4 smaller doses of E. acervulina oocysts at 2 or 3 week intervals would more closely approximate field conditions of cyclic reinfection and may bring out variations between groups of chicks with respect to duration of immunity. It

would be helpful to monitor weight gains and oocyst production following each exposure.

It is also possible that more advanced MD involvement would have a greater effect upon the immune system of the chick. Burg et al. (1971) were able to demonstrate immune suppression in MD infected chicks before lesions were formed but Rice and Reid (1973) showed that the degree of suppression was proportional to the level of MD involvement. While this study did not show any effect of an early MD involvement upon resistance to E. acervulina, more advanced MD may cause a more severe immune suppression. Inoculation with MDHV at two weeks of age was recommended for this study by Dr. Rude of Salsbury Laboratories. His studies had shown that when the MDHV isolate used in this study was inoculated into one day old chicks, mortality was high by three weeks. The older chicks were when inoculated, the less severe were the morbidity and mortality. More advanced MD could be achieved in further studies by inoculating chicks with MDHV earlier and/or waiting longer before introducing the E. acervulina oocysts.

CONCLUSIONS

The present study was designed to detect any effects of either MDHV or HVT upon the ability of young chickens to resist infection upon primary infection with E. acervulina and to develop immunity to reinfection. Groups of chicks

were inoculated with either MDHV or HVT. These chicks plus a group of control chicks were inoculated with a series of 20 inoculations with E. acervulina oocysts. Weight data did not indicate any difference in the response of either virus group as compared to the controls. These chicks were subsequently inoculated with a massive dose of E. acervulina oocysts. Again, there was no significant variation of either virus group from the controls based on weight gains, oocyst recovery from feces, or intestinal lesion scoring at autopsy.

Based on this study, therefore, it is concluded that neither MDHV nor HVT has any effect upon immunity to E. acervulina in young chickens. There is no indication that vaccination of one day old chicks with HVT as a prophylaxis against MD renders chicks more susceptable to E. acervulina infection.

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APPENDIX

Table 3. Weight in grams of chicks in group A on days indicated during administration of immunizing inoculations of *E. acervulina* oocysts.

CHICK NO.	WEIGHT OF CHICK ON DAY INDICATED						
	15	18	21	24	27	30	33
A1	155	175	175	185	215	230	255
A2	135	155	180	200	240	260	300
A3	150	185	200	225	270	290	330
A4	135	160	170	200	240	270	315
A5	155	190	215	235	280	315	355
A6	120	150	165	185	225	260	290
A7	160	195	225	250	305	350	380
A8	125	150	165	175	225	245	275
A9	150	175	200	230	285	315	355
A10	140	160	185	210	250	285	320
A11	145	180	200	220	255	285	310
A12	145	175	210	235	260	300	340
A13	120	140	150	160	200	215	250
A14	150	180	200	220	260	280	310
A15	145	175	195	220	260	300	340
A16	150	180	200	235	275	305	350
A17	130	155	170	190	225	255	285
A18	175	205	205	220	260	305	340
A19	150	180	210	240	280	320	355
A20	135	155	170	200	220	255	285
A21	150	185	200	220	260	295	325
A22	130	155	180	200	230	280	295
A23	140	165	185	200	240	270	310
A24	115	135	145	160	200	225	255
A25	150	175	200	230	280	320	375
A26	145	175	200	225	270	300	340
A27	130	160	170	200	240	270	300
A28	170	205	215	255	310	340	380
A29	145	170	190	210	250	285	315
A30	140	160	165	180	220	250	280

Table 4. Weight in grams of chicks in group B on days indicated during administration of immunizing inoculations of E. acervulina oocysts.

CHICK NO.	WEIGHT OF CHICK ON DAY INDICATED 15	18	21	24	27	30	33
B1	115	140	160	190	230	255	285
B2	160	195	225	260	310	355	385
B3	130	160	185	210	245	275	310
B4	115	135	145	160	195	210	230
B5	125	160	180	195	235	265	300
B6	120	145	145	150	175	195	215
B7	125	160	180	200	245	280	315
B8	115	145	160	190	230	255	280
B9	125	150	170	190	220	245	280
B10	120	150	165	185	220	240	270
B11	120	155	165	180	230	240	275
B12	135	160	180	190	235	255	280
B13	120	140	155	185	230	260	290
B14	145	170	195	215	255	285	305
B15	145	175	190	205	250	275	310
B16	105	135	165	190	240	270	305
B17	125	150	175	190	230	255	300
B18	135	170	195	210	260	285	330
B19	125	155	170	185	215	245	275
B20	120	150	165	180	225	240	265
B21	130	160	175	190	230	250	280
B22	135	165	175	190	230	250	285
B23	120	150	155	165	210	235	270
B24	115	140	145	165	200	220	250
B25	130	160	165	190	220	245	275
B26	145	170	180	190	230	255	290
B27	110	140	155	170	210	230	260
B28	135	170	190	220	255	285	320

Table 5. Weight in grams of chicks in group C on days indicated during administration of immunizing inoculations of E. acervulina oocysts.

CHICK NO.	WEIGHT OF CHICK ON DAY INDICATED 15	18	21	24	27	30	33
C1	120	155	160	185	220	250	285
C2	140	175	185	220	260	295	340
C3	140	165	180	195	235	250	280
C4	145	165	180	190	230	250	280
C5	130	150	170	180	210	240	270
C6	120	140	150	160	190	215	230
C7	150	185	205	220	255	275	300
C8	120	145	170	205	240	285	330
C9	135	165	175	190	220	245	280
C10	120	150	160	180	220	240	270
C11	135	155	175	190	240	275	320
C12	140	170	200	210	245	280	320
C13	135	160	170	185	230	260	280
C14	140	170	180	200	235	265	300
C15	130	160	165	185	230	260	300
C16	140	170	195	220	260	300	340
C17	135	165	175	200	245	280	315
C18	120	145	155	160	210	245	275
C19	140	170	185	210	250	280	320
C20	155	190	215	240	295	330	370
C21	145	175	200	210	260	280	320
C22	140	165	190	210	250	285	320
C23	135	165	185	210	255	285	330
C24	140	170	185	205	245	280	310
C25	145	170	185	200	245	275	310
C26	135	160	170	200	240	270	305
C27	125	150	160	175	210	240	270
C28	130	150	170	200	245	275	310
C29	125	155	170	190	225	250	280
C30	130	160	180	200	235	265	300

Table 6. Weight in grams of chicks in control group D on days indicated during administration of immunizing inoculations of E. acervulina oocysts to groups A, B, and C.

CHICKS NO.	WEIGHT OF CHICK ON DAY INDICATED 15	18	21	24	27	30	33
D1	130	155	190	215	255	280	305
D2	140	170	200	240	275	305	330
D3	130	150	190	220	270	300	325
D4	125	145	175	205	250	275	300
D5	130	150	170	200	235	255	275
D6	125	145	160	180	205	220	240
D7	130	150	170	195	230	245	275
D8	135	150	180	200	225	240	270
D9	150	180	220	255	295	330	355
D10	135	160	195	230	275	300	340
D11	130	150	180	215	250	275	300
D12	140	160	195	210	250	270	305
D13	140	165	195	225	255	275	305
D14	145	160	185	200	220	230	245
D15	135	160	200	225	270	300	335
D16	145	160	190	215	250	265	295
D17	140	170	200	240	280	310	335
D18	150	170	200	225	260	290	320
D19	105	125	140	150	165	180	200
D20	130	145	180	210	250	265	295
D21	140	170	195	235	280	305	340
D22	135	160	195	230	270	300	325
D23	130	145	175	205	245	265	295
D24	155	180	220	250	290	325	350
D25	165	195	235	270	310	350	375
D26	145	165	190	220	250	275	305
D27	145	165	195	230	265	280	315
D28	130	155	190	225	260	285	305
D29	140	165	200	230	270	295	330
D30	150	175	210	245	290	315	345

Table 7. Weight in grams of chicks in control group E on days indicated during administration of immunizing inoculations of E. acervulina oocysts to groups A, B and C.

CHICKS NO.	WEIGHT OF CHICK ON DAY INDICATED 15	18	21	24	27	30	33
E1	120	140	165	190	220	240	265
E2	140	175	215	250	295	325	365
E3	130	160	200	230	270	315	340
E4	130	150	185	220	240	285	315
E5	150	185	225	270	310	350	385
E6	135	160	200	230	270	300	330
E7	145	165	205	230	255	300	340
E8	150	185	225	260	290	335	375
E9	125	155	200	235	270	315	355
E10	140	155	190	210	245	275	300
E11	140	160	205	235	280	310	335
E12	135	155	190	210	245	265	295
E13	130	150	175	200	225	240	260
E14	125	150	185	210	245	275	305
E15	120	145	175	210	245	270	300
E16	145	170	205	235	265	300	330
E17	140	170	205	230	275	305	340
E18	135	160	195	225	265	300	325
E19	135	160	200	220	255	290	310
E20	140	170	215	245	275	310	350
E21	135	165	200	235	265	305	335
E22	145	165	200	220	255	290	320
E23	145	160	200	230	265	290	320
E24	140	160	195	215	240	265	300
E25	105	125	145	160	190	210	230
E26	130	150	185	215	245	275	300
E27	135	155	190	215	240	270	300
E28	140	160	205	240	280	320	350
E29	125	150	170	195	225	260	290
E30	135	160	190	210	225	250	285

Table 8. Weight in grams of chicks in group A following administration of E. acervulina oocyst challenge.

CHICK NO.	WEIGHT OF CHICK ON DAY INDICATED							
	43	44	45	46	47	48	49	50
A16	485	500	520	525	545	560	*	*
A17	390	405	420	425	440	460	*	*
A18	480	480	500	500	515	530	*	*
A19	480	500	520	530	540	555	*	*
A20	395	400	410	420	425	435	*	*
A21	480	485	500	520	535	545	565	595
A22	395	400	415	420	435	445	455	485
A23	445	450	470	485	500	505	515	545
A24	345	350	370	375	385	395	405	430
A25	520	530	550	560	590	600	610	635
A26	465	500	510	525	545	540	560	590
A27	430	435	455	460	480	490	495	520
A28	530	545	570	585	600	615	620	650
A29	435	450	470	480	490	505	510	535
A30	400	410	425	435	455	460	470	490

*Not weighed. Chick had been killed and autopsied.

Table 9. Weight in grams of chicks in group B following administration of E. acervulina oocyst challenge.

CHICK NO.	WEIGHT OF CHICK ON DAY INDICATED							
	43	44	45	46	47	48	49	50
B16	460	465	490	500	515	535	*	*
B17	420	425	450	465	475	495	*	*
B18	460	460	490	500	515	530	*	*
B19	415	420	435	455	470	490	500	520
B20	400	410	430	450	460	475	495	520
B21	400	405	420	435	445	460	470	490
B22	420	420	440	455	470	480	485	515
B23	380	390	405	410	420	430	445	455
B24	345	345	360	370	380	390	405	410
B25	400	410	415	435	445	460	470	485
B26	430	440	460	475	495	505	520	540
B27	380	375	395	425	430	440	455	470
B28	450	450	470	495	515	525	540	565

*Not weighed. Chick had been killed and autopsied.

Table 10. Weight in grams of chicks in group C following administration of E. acervulina oocyst challenge.

CHICK NO.	WEIGHT OF CHICK ON DAY INDICATED							
	43	44	45	46	47	48	49	50
C16	490	500	520	530	550	565	*	*
C17	460	475	495	505	520	525	*	*
C18	420	425	445	455	465	480	*	*
C19	440	455	465	475	490	500	*	*
C20	535	545	565	575	600	615	*	*
C21	435	445	460	460	480	490	500	520
C22	465	470	490	500	520	540	550	570
C23	460	465	480	485	515	510	540	570
C24	445	455	480	480	500	520	530	560
C25	450	455	470	490	510	525	540	565
C26	445	455	470	485	500	515	530	545
C27	400	410	430	440	455	470	480	495
C28	410	435	460	485	480	500	500	525
C29	405	410	430	445	460	470	480	495
C30	425	430	440	445	460	465	480	495

*Not weighed. Chick had been killed and autopsied.

Table 11. Weight in grams of chicks in group D following administration of E. acervulina oocyst challenge.

CHICK NO.	WEIGHT OF CHICK ON DAY INDICATED							
	43	44	45	46	47	48	49	50
D16	405	405	420	430	420	405	*	*
D17	455	460	480	485	460	440	*	*
D18	415	420	425	435	430	425	*	*
D19	275	280	290	295	290	280	*	*
D20	410	405	420	425	390	Dead		
D21	470	480	500	510	490	480	485	520
D22	455	460	475	490	465	460	480	505
D23	400	410	425	440	420	415	430	450
D24	485	490	510	530	510	500	520	545
D25	520	525	545	555	540	535	545	575
D26	400	410	420	430	415	410	410	440
D27	435	445	460	465	450	450	480	505
D28	420	430	435	445	440	430	460	475
D29	450	460	465	470	460	465	475	510
D30	490	500	525	540	510	510	505	550

*Not weighed. Chick had been killed and autopsied.

Table 12. Weight in grams of chicks in control group E following administration of E. acervulina oocyst challenge to groups A, B, C and D.

CHICK NO.	WEIGHT OF CHICK ON DAY INDICATED							
	43	44	45	46	47	48	49	50
E16	460	465	485	500	520	530	*	*
E17	480	470	490	510	530	535	*	*
E18	455	455	475	490	505	510	*	*
E19	440	440	460	465	480	490	*	*
E20	500	500	520	530	560	575	*	*
E21	480	490	500	520	530	550	565	585
E22	460	460	480	495	510	525	545	570
E23	450	455	475	500	515	525	550	565
E24	410	420	445	450	480	480	500	510
E25	325	335	350	355	360	365	385	390
E26	420	425	435	450	465	475	500	510
E27	440	440	465	475	490	500	515	540
E28	480	480	500	525	540	555	570	590
E29	410	420	435	440	460	470	490	510
E30	400	395	415	430	445	455	470	485

*Not weighed. Chick had been killed and autopsied.

Table 13. Data from E. acervulina oocyst isolation from 24-hour fecal accumulations from groups A, B, C and D on days 48 and 49.

Group	Chick Nos.	Volume	Dilution Factor	Counts*	Oocysts/ml	Oocysts/Chick
			(DAY 48)			
A	1-5	21 ml	1	16/9,18/9	1.7×10^5	7.14×10^5
	6-10	23 ml	1	6/9, 6/9	6.0×10^4	2.76×10^5
	11-15	22 ml	1	30/9,29/9	2.9×10^5	1.30×10^6
B	1-5	28 ml	1	4/9, 8/9	6.0×10^4	3.36×10^5
	6-10	31 ml	1	8/9, 6/9	7.0×10^4	4.34×10^5
	11-15	17 ml	1	1/9, 3/9	2.0×10^4	6.80×10^4
C	1-5	13 ml	1	21/9,22/9	2.2×10^4	5.59×10^5
	6-10	21 ml	1	6/9, 9/9	7.5×10^4	3.15×10^5
	11-15	18 ml	1	2/9, 5/9	3.0×10^4	1.08×10^5
D	1-5	55 ml	10	56/5,64/5	1.08×10^7	1.19×10^8
	6-10	54 ml	10	61/5,56/5	1.09×10^7	1.18×10^8
	11-15	48 ml	10	55/5,64/5	1.07×10^7	1.03×10^8
			(DAY 49)			
A	6-10	26 ml	1	2/9, 0/9	1.0×10^3	5.20×10^4
	11-15	36 ml	1	0/9, 1/9	5.0×10^3	3.60×10^4
B	6-10	34 ml	1	1/9, 2/9	1.5×10^3	1.02×10^4
	11-15	44 ml	1	0/9, 1/9	5.0×10^3	4.40×10^4
C	6-10	20 ml	1	4/9, 3/9	3.5×10^4	1.40×10^5
	11-15	34 ml	1	2/9, 3/9	2.5×10^4	1.70×10^5
D	6-10	46 ml	10	33/5,37/5	6.3×10^6	5.80×10^7
	11-15	51 ml	10	29/5,31/5	5.4×10^6	5.51×10^7

*numerator = number of oocysts counted,
denominator = number of large squares counted

Table 14. Autopsy data for all chicks indicating E. acervulina and MD involvement.

Group	E. acervulina lesion scores					MD lesions				
	A	B	C	D	E	A	B	C	D	E
Chick										
1	0	0	0	4	0	-	-	-	-	-
2	0	0	0	3	0	-	-	-	-	-
3	0	0	0	4	0	-	-	-	-	-
4	0	0	0	4	0	-	-	-	-	-
5	0	0	0	3	0	-	-	-	-	-
6	0	0	0	3	0	-	-	-	-	-
7	0	0	0	4	0	-	-	-	-	-
8	0	0	0	4	0	-	-	-	-	-
9	0	1	0	4	0	V	-	-	-	-
10	0	0	0	4	0	-	-	-	-	-
11	0	0	0	3	0	-	-	-	-	-
12	0	0	0	4	0	V	-	-	-	-
13	0	0	0	4	0	-	-	-	-	-
14	0	0	0	4	0	-	-	-	-	-
15	0	0	0	4	0	-	-	-	-	-
16	0	0	0	4	0	V,S	-	-	-	-
17	0	0	0	4	0	-	-	-	-	-
18	0	0	0	4	0	-	-	-	-	-
19	0	0	0	4	0	-	-	-	-	-
20	0	0	0	4	0	-	-	-	-	-
21	0	0	0	3	0	-	-	-	-	-
22	0	0	0	3	0	V	-	-	-	-
23	0	0	0	4	0	-	-	-	-	-
24	0	0	0	3	0	-	-	-	-	-
25	0	0	0	3	0	V	-	-	-	-
26	0	0	0	3	0	-	-	-	-	-
27	0	0	0	4	0	-	-	-	-	-
28	0	0	0	4	0	-	-	-	-	-
29	0	*	0	3	0	-	*	-	-	-
30	0	*	0	4	0	-	*	-	-	-

*Group B contained only 28 chicks.
- = no visible lesions
V = vagus nerve
S = sciatic nerve